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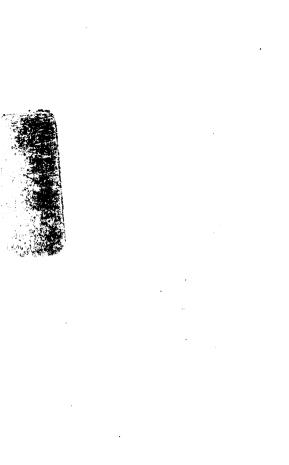
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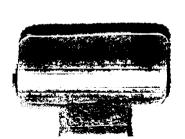
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The Demonstration of Spirochæta Pallida in Lesions of Acquired Syphilis.

BY ALEXANDER A. UHLE, M.D., AND WM. H. MACKINNEY, M.D.

OF the various methods employed to establish the etiologic relation of Spirochæta pallida to syphilis, those methods dealing with its demonstration in secretions of syphilitic lesions, or in the fluids obtained from them by artificial means, have received the most attention. Schaudinn based his discovery upon positive findings in 26 cases of syphilis, the secretions being examined in a fresh state.

According to his description, the organism varies in length from 4 to 14 microns and its width does not exceed 0.5 micron. Its ends are pointed and it shows three to twelve spiral curves. It is actively motile, showing both a rotatory and longitudinal motion. Flagella have been demonstrated at either extremity.

Hoffmann, shortly after, demonstrated a spiral organism in the fluids obtained from the liver, spleen, inguinal glands, and pemphi-

goid eruption of a child dying of hereditary syphilis, which was in all respects similar to that described by Schaudinn.

Since this discovery, numerous observers have confirmed their finding by similar or slightly different technique. In all of these observations the findings were so constant that at the present time the causal factor of syphilis is conceded by many observers to be Spirochæta pallida, Treponema pallida, or Spironema pallida, as it is variously called.

The first attempts made to demonstrate spirochætæ in the syphilic tissue were made by Berterelli and Volpino, who succeeded in demonstrating the organism in the liver and spleen of children dying of hereditary syphilis. Their method consisted in treating very thin sections of tissue to a twenty-four to forty-eight hours' immersion in a 0.5 per cent. solution of silver nitrate, followed by washing in a solution of pyrogallic acid and sodium acetate. They remain in this solution for fifteen minutes and are then returned to the silver solution, in which they remain until they take on a yellowish-brown color. They do not give their method in detail.

In this manner they succeeded in demonstrating Spirochæta pallida, it taking a dark-brown, almost black stain. They guarded their findings with comparisons of normal tissue.

Levaditi modified the Ramoan y Cajal method for nerve fibers, and formulated an exact method for staining syphilitic tissue *in toto*, with a view of determining the presence or absence of spirochætæ. His method has been repeatedly employed and has given satisfactory results.

It is as follows: Small pieces of tissue, not more than 2 mm. in thickness, are hardened in 10 per cent. formalin for twenty-four hours; they are then placed in 95 per cent. alcohol for the same period, and washed in water for a few hours.

Specimens are then transferred to a freshly made solution of silver nitrate (1.5 per cent.) for three successive days, changing the solution each day, and maintaining a blood temperature. Light must be excluded. This is best accomplished by placing the bottles in the incubator.

From the silver solution they are transferred to a 2 per cent. solution of pyrogallic acid, to which is added 5 per cent. of formalin.

This is also kept in the dark, but at room temperature. They are then passed through 85 per cent., 95 per cent., and absolute alcohol, embedded in paraffin and cut at about 5 microns.

By this method he succeeded in demonstrating Spirochætæ pallida in the initial lesion of syphilis and in secondary papules. Syphilitic tissue from apes inoculated with syphilis also gave positive results, as did the organs of children dying of hereditary syphilis.

A. Buschke and W. Fischer (Berl. klin. Woch.) employed this method successfully in demonstrating Spirochætæ pallida in a chancre and in secondary papules, without noting any particular changes in the histologic appearance of the tissue. Their most interesting discovery, however, was the finding of this organism in the heart muscle, as well as in the remaining organs of a child dying of hereditary syphilis. In this case the spirochætæ had been found in large numbers before death in the fluid obtained from an artificial cantharidal blister.

In a second article by Levaditi and Manouélian (Compt.-rend. Soc. de biol., Paris, 1906) the more exact findings regarding the occurrence of Spirochætæ pallida in the primary and secondary lesions of syphilis are described. Their deductions are drawn from a study of six doubtless syphilitic lesions. They were able to demonstrate this organism in two initial lesions and in a secondary papule from the anterior belly wall. From these studies they conclude that spirochætæ reach the deeper tissues by way of the blood and lymph channels, and their appearance in large numbers in the walls and lumen of bloodvessels explains the endarteritis and periarteritis so constant in syphilis.

In six lesions removed from apes artificially inoculated with syphilis, they report positive findings in two cases, Spirochætæ pallida being found in the periphery of the ulceration, in the skin papillæ, in the wall and lumen of the bloodvessels, and in the deeper layers of the epithelium. They were unable to demonstrate organisms in the depths of the tissue.

Burent and Vincent (Compt.-rend. Soc. de biol., Paris, 1906) demonstrated Spirochætæ pallida in the chancre, finding many organisms in the papillæ, and in the subepithelial connective tissue. They occurred in lymph channels and in the thickened vessel walls.

Veiklon and Girard report the findings in the roseola of syphilis. They found large numbers of Spirochætæ pallida in the capillaries of the papillæ and in the vessels beneath. From their examinations the authors conclude that the roseola is not due to a toxic activity but to embolism in the vessels of the skin papillæ, which provokes a congestive hyperemia, and perivascular infiltration similar to the roseola of typhoid. They also report positive findings in the white pneumonias of two cases of hereditary syphilis, in the adrenals of another case, and in both the maternal and fetal portions of the placenta.

Finger reports positive findings in two chancres, one being of the gangrenous type.

Ehrmann (Dermat. Zeits: h., Berlin, 1906) found Spirochætæ pallida between the epithelial cells in non-ulcerated secondary lesions. The organisms were most abundant above the stratum granulosum, although present in all layers. In the papillæ they were found in greatest number, and he offers the hypothesis that those in the epithelium are extruded from the papillary bloodvessels. They are always found between the cells and never within them. He therefore concludes that not only are moist and ulcerated lesions to be considered contagious, but the dry forms as well, and should therefore be handled with great care.

E. Hoffmann and Beer (Deutsch. med. Woch., 1906) call attention to a method of staining which can be carried out in less time than the Levaditi method, but the results they have obtained are no more satisfactory than those obtained by the older method. This fact is also noted by subsequent writers who employed this method, which is the following: Fixation in formalin (10 per cent.) for twenty-four hours; alcohol (95 per cent.) for fifteen hours; immersion in a 1.5 per cent. solution of silver nitrate, to which is added 10 c.c. of pure pyridin, for three hours at the room temperature, and a further three hours in the paraffin closet at a temperature of 40° to 50° C. Light must be excluded.

They are then placed in a solution made by taking 90 c.c. of a 4 per cent. solution of pyrogallic acid, to which 10 c.c. of pure acetone is added, and to 85 c.c. of this solution 15 c.c. of pure pyridine is added. The tissue remains in this solution over night, light being excluded. The tissue is then embedded and cut. Sections can be counterstained

with polychrome methylene blue. By this method they succeeded in demonstrating Spirochætæ pallida in a syphilitic bubo during the secondary period of incubation and in an orbicular syphilide.

A. Blaschko (Med. klin. Woch., 1906) used the Levaditi method in the study of four chancres and one condyloma. In all of these cases he reports positive findings, Spirochætæ pallida occurring in the epithelium, in the papillæ, and in the connective-tissue spaces. He found them in great numbers in the walls of bloodvessels and in their immediate vicinity. It is interesting to note that one of his specimens had remained in formalin for a year.

In July, 1906, Dr. Karl Reuter (Zeit. j. Hygiene u. Infectionskr.) reports several very interesting observations. He examined the tissue of a child dying of hereditary syphilis, the tissues having been preserved in formalin since 1903. The lesions included a diffuse white pneumonia, a gumma of the upper lobe of the right lung, and an interstitial pancreatitis. In all of the sections he had positive findings. In the gumma of the lung large numbers of Spirochætæ pallida were found at the periphery, underlying the limiting membrane. He could not demonstrate them in the central necrotic area. In the white pneumonic areas they occurred in less number, but were more evenly distributed. In the pancreas they were found in the interstitial connective tissue.

In a subsequent study of a recent gumma of the testicle he was unable to find spirochætæ, and cites as an analogy the difficulty of demonstrating tubercle bacilli in caseous lesions.

A series of experiments was carried out to determine the influence of mercury upon the numbers of Spirochætæ pallida found. With this object he studied three cases of hereditary and one case of acquired syphilis. In them he found it very difficult or impossible to demonstrate Spirochæta pallida after mercury had been ingested for a short time. In all of them large numbers were found prior to treatment, the examinations being made from smears of existing lesions.

The same author made studies to determine the presence of Spirochætæ pallida in cases of specific aortitis. In a patient dying suddenly of embolism, with no knowledge of syphilitic infection, and having taken no mercury, he was able to demonstrate Spirochætæ pallida in the wall of the aorta, particularly beneath the intima. This dis-

covery is of great value in explaining the arteritis of the Döhle-Heller type.

As to the findings in tertiary lesions they are almost entirely negative. We have been unable to find positive reports in gummatous or tuberculous syphilitic lesions.

E. Tomasczewski examined the scrapings from ten tuberculous syphilides, cover-slips being made and stained by the Giemsa stain. In five of these patients he reports the presence of Spirochætæ pallida. By this method, however, the opportunity for error is so great that they can have little positive value.

Personal Observations. During the past four months we have been engaged in the study of syphilitic tissue, with the object of determining the presence or absence of Spirochætæ pallida in the lesions of acquired syphilis. In selecting the specimens care was taken to include with preference only non-ulcerated and non-eroded lesions. This was possible with the majority of papular syphilides, and to a limited extent with chancres. In tuberculous lesions the periphery was selected at a point showing the least ulceration, the object being to avoid contaminations of spiral organisms.

The method of Levaditi was employed, and we believe it to be the best method for staining the tissue *in toto*. The specimens were removed from twenty-four patients suffering with acquired syphilis, the lesions including chancres, various papular lesions, tuberculous syphilides, and one gumma.

As controls lesions of chancroids, chancroidal buboes, psoriasis, scabies, acne vulgaris, varicella, and venereal vegetations were studied.

Normal skin taken from fifteen individuals was stained in the same manner and studied.

The following is a report of the findings in each case.

CASE I.—J. D. Syphilis of five months' duration. Was taking potassium iodide for two months prior to examination. There is still induration present at the site of the chancre. No syphilitic lesions were present. Has an iodide acne on the back, one being removed and examined. No spirochætæ were found.

Case II.—W. U. Chancre in sulcus. Fading, slightly pigmented macular eruption on back and chest. Macule removed from back. No spirochætæ found.

CASE III.—A. H. Chancre three months ago. Profuse general eruption of large papulosquamous syphilides. No treatment. Epithelium intact. A few spirochætæ between the epithelial cells and many in the cellular infiltrate.

CASE IV.—H. P. Eruption of five days' duration. History of sore on penis four weeks ago. Profuse maculopapular syphilide. Marked round-cell infiltration, but no spirochætæ found.

CASE V.—H. T. Chancre in sulcus. Discrete papular syphilide. No treatment. Spirochætæ pallida found in moderate numbers in the papillæ and in the connective-tissue lymph spaces. After five weeks' treatment by mercury in pill form, no spirochætæ were found in a pigmented portion of skin removed from the back, marking the site of a syphilitic papule.

CASE VI.—W. H. A. History of chancre seven months ago. Was treated at once for two months with pills, and then irregularly until five weeks ago. For the past five weeks no treatment. Now has a discrete papular syphilide on the back and mucous patches on the tongue and lip. Papule removed from back. No spirochætæ were found.

CASE VII.—A. H. Syphilis of eighteen months' duration. Has been taking mercury and potassium iodide. Has an iodide acne, one lesion of which was removed. No spirochætæ were found.

CASE VIII.—J. C. Chancre and general syphilitic macular eruption. Was circumcised and a part of the chancre, the skin of the prepuce, together with a mucous patch from the scrotum and a macule from the leg, were examined. Spirochætæ pallida found in moderate numbers in the chancre, mucous patch, and macule. Not found in the skin of the prepuce.

CASE IX.—G. A. Syphilis of three months' duration. Has neglected treatment. Took eight mercurial inunctions and then discontinued treatment until eighteen days ago, when he began to take mercury in pill form. A fading maculopapular eruption is still present, a papule being removed. No spirochætæ were found, and very slight round-cell infiltration.

CASE X.—J. Mc. Has been admitted to the Philadelphia General Hospital three times in the past eight years for the treatment of recurrent tuberculous syphilides. Has a large lesion on the left arm,

a piece of which, together with the healthy adjacent skin, was removed. He has taken no treatment for the past four years. Spirochætæ present in moderate numbers. They occur in the deeper connective tissue some distance from the ulcerated surface and lying free in the lymph spaces. The adjacent skin contained no spirochætæ.

CASE XI.—W. G. Chancre removed by circumcision and syphilitic papule removed from back. Spirochætæ pallida present in the chancre. In the papule the epithelium is intact. Spirochætæ pallida found in the papillæ and in the lymph spaces of the corium.

A pigmented portion of skin marking the site of a papule was removed after the patient had taken mercury in pill form for nine weeks. Examination showed no spirochætæ or cellular infiltration, the section appearing as normal skin.

CASE XII.—J. W. Syphilitic papule removed from back. Had been taking mercury in pill form for three days. Spirochætæ present in small numbers between the cellular infiltrate and within the lymph spaces.

CASE XIII.—P. Mc. Chancre obtained by circumcision. A maculopapular syphilide removed from back and from arm. No treatment. Chancre shows pronounced round-cell infiltration. No spirochætæ found in epithelium. The bloodvessel walls are thickened, especially the media and adventitia. A number of spirochætæ found diffusely in the tissue of the chancre and the papule of the arm, but none were found in that removed from the back.

CASE XIV.—J. F. Chancre removed by circumcision and syphilitic papule removed from back. No treatment. Chancre shows marked round-cell infiltration and a moderate number of spirochætæ. The papule from the back shows but slight round-cell infiltration, and spirochætæ were demonstrated only after a most diligent examination.

CASE XV.—Syphilis of several years' duration. Portion of periphery of tuberculous syphilide removed from leg. No spirochætæ were found.

CASE XVI.—Syphilis of nine months' duration. Neglected treatment. Recurrent eruption, mucous patches in mouth and anal condylomas. Was circumcised and a portion of the skin of the prepuce, together with a condyloma of the anal region, examined. Spirochætæ were found in the condyloma but not in the skin of the prepuce.

CASE XVII.—B. R. Treated for syphilis by mercury in pill form for eight days. Prepuce removed by circumcision; a mucous patch on it and a condyloma of the anus examined. Spirochætæ were found in the mucous patch of the prepuce, but not in the condyloma.

CASE XVIII.—Chancre removed by circumcision and a syphilitic papule taken from the buttocks. No treatment. No spirochætæ were found in the chancre and the sections of the papule were also negative.

CASE XIX.—J. B. Profuse maculopapular eruption present over entire body. No treatment. Spirochætæ pallida found in large numbers in a papule removed from the back. A great number were present in the skin papillæ, and some sections showed similar organisms between the epithelial cells.

CASE XX.—J. K. Syphilis of fourteen months' duration. Has a large tuberculous syphilide on the right leg; a portion at the periphery of the lesion and the adjacent healthy skin were examined. In one section a few spirochætæ were found lying free in lymph spaces, and in another, after a careful search, they were demonstrated in the depth of the tissue.

CASE XXI.—J. R. Chancre removed by circumcision and a papule taken from the side of the chest. Section of the chancre shows pronounced round-cell infiltration and a moderate number of spirochætæ lying between the cells. The epithelium of the papule is intact; no spirochætæ between the cells. There is a moderate infiltration of round cells and a small number of spirochætæ diffusely distributed between the cells, a few occurring around the capillaries of the papillæ.

CASE XXII.—Syphilis of four years' duration. Portion of tuberculous syphilide removed from the leg. Specimen shows connectivetissue hyperplasia and moderate amount of cellular infiltrate. No spirochætæ were demonstrable.

CASE XXIII.—H. P. Has two well-defined indurated ulcers on the reflected layer of the prepuce. Patient was observed for ten weeks and is still under observation, but shows no evidence of a secondary eruption on the body. At the end of ten weeks distinct mucous patches occurred on the glans penis. Prepuce was removed by circumcision and a piece of normal skin from the back. No spirochætæ were found in sections of the chancre, the skin of the prepuce or back.

CASE XXIV.—Through the kindness of Dr. A. O. J. Kelly a specimen of gumma of the brain was obtained directly from the autopsy table. A history of this patient states that he was unaware of a syphilitic infection and had never knowingly taken antisyphilitic treatment. A portion of the gumma removed from the periphery shows spirochætæ identical with those found in primary and secondary lesions of syphilis. They are present in large numbers at the periphery; the remaining tissue is necrotic.

Four lesions of psoriasis, from different patients, showed hyperplasia of the connective tissue, but nothing resembling spirochætæ. Tissue from six cases of scabies showed only a slight degree of cellular infiltration and separation of the connective tissue due to serous exudation. Three lesions of varicella from one patient showed necrotic epithelium and serous exudation. A short organism resembling a bacillus, about 4 microns in length, was found lying between the connective-tissue bundles. Nothing resembling the spirochætæ was found. Venereal vegetations from three patients showed marked papillary and connective-tissue hyperplasia, but no spirochætæ. Fifteen sections of normal skin from different individuals were examined with negative results. Nine chancroids and two chancroidal buboes were submitted to a careful examination, but with negative results.

It will be seen that observations were made upon 24 patients; subjects of acquired syphilis, from whom 34 pieces of tissue were examined. They include 7 chancres, 19 cutaneous secondary lesions, together with 3 pieces of healthy-looking skin from patients with lesions of syphilis, 4 tubercular syphilides, and 1 gumma.

In these 24 cases positive findings were obtained in 14 and negative in 10. In the patients giving a negative result 7 had received antisyphilitic treatment and 3 had not. It is the consensus of opinion that Spirochætæ pallida are not found after mercury has been taken for some time. This has been the case with the patients we have examined, the organism disappearing before the cutaneous manifestations. The time of the disappearance of the organism after the ingestion of mercury is not known. In two cases in which spirochætæ were found before the taking of mercury, none could be demonstrated after five and nine weeks of mercurial treatment. They were found in one patient after taking mercury for three days, and in one

after eight days. The seven patients in whom the examinations were negative had been taking treatment from seventeen days to eighteen months.

With a few exceptions it required a careful and diligent search to determine the presence of this organism. One is very likely at times to be confused by the similar appearance of elastic tissue, and we are led to believe that observers who report the finding of large numbers of spirochætæ in the walls of bloodvessels may have made this mistake.

Spirochæta pallida is always the same no matter in what tissue it may be found, but the morphology may vary somewhat, depending upon the character and age of the lesion. Staining of tissue *in toto* is not the best means of demonstrating its morphology, and in estimating its length the cutting of the section must always be considered.

In recent lesions the organism is distinctly spiral, taking a black stain, showing from four to twelve curves, and is clear cut. In tertiary lesions it seems to lose some of its distinctness of outline and assumes a more wavy appearance. It may be found isolated or grouped in small or large numbers, and, as a rule, occurs in largest quantities in the lymph spaces. It may be demonstrated in the epithelium, but not constantly.

Spirochætæ have never been demonstrated in the healthy skin, even where this was taken directly adjoining a syphilitic lesion.

From the findings one must consider all lesions of syphilis contagious and handle them with due care.

As far as our observations go, spirochætæ are not found in other than syphilitic eruptions, occurring in subjects of syphilis.

In conclusion, we may say that the frequency with which this organism has been demonstrated in syphilitic lesions, by numerous observers, the results of inoculation experiments upon apes, and the relation which this organism bears to mercury speak in no uncertain terms for its specificity in the causation of syphilis.

We desire to thank Dr. A. O. J. Kelly for extending to us the facilities of the German Hospital Laboratory and for many courtesies extended in the preparation of this paper

We are greatly indebted to Mr. Karl Becker for valuable assistance in the preparation of the specimens.

January 10, 1907.

A Bacteriological and Histological Study of Organs Removed from Tuberculous Subjects.

BY RANDLE C. ROSENBERGER, M.D., PHILADELPHIA.

(From the Laboratories of the Jefferson Medical College Hospital.)

Most of the material collected for this report was obtained from cases which came to autopsy at the Henry Phipps Institute during the writer's service there as pathologist.

The cases, with but one or two exceptions, were instances of chronic ulcerative tuberculosis, some with small and others with massive cavity formation.

The object in studying these organs was more for personal gratification than for general information, but the findings were so interesting that a presentation of the work does not seem improper. The general idea is gaining ground that when a person is affected with pulmonary tuberculosis the bacilli are spread broadcast by means of the blood or lymph, or perhaps both, when they lodge in different organs, bringing about a general systemic or visceral infection. This view seems almost positively borne out by the results of the studies about to be recorded.

The organs studied were the heart, liver, spleen, intestines, appendix, thoracic duct, and bloodvessels. The tissues were all infiltrated with paraffine; stained for histological study with hematoxylin and Van Giesen, and for tubercle bacilli with carbol fuchsin, followed with the mixture of sweet spirits of nitre and saturated alcoholic solution of malachite green.

Of 62 livers, 20 were diagnosticated macroscopically as fatty infiltration, 18 as chronic or passive congestion, 2 showed miliary tuberculosis, 1 calcified tubercles, while 2 showed amyloid infiltration. The remaining 19 organs did not show any appreciable pathological change, yet sections were stained with quite surprising results. Fifty-two livers, or 83.8 per cent., showed miliary tubercles. Some of these histological tubercles consisted only of lymphoid elements, others contained typical giant and epithelioid cells, and others showed caseation. It will be noticed that, upon gross examination, tuberculosis

was diagnosticated only three times. The tubercles were situated deeply in the parenchyma, and none were found in the capsule of Glisson. As regards the intralobular or interlobular distribution, the tubercles were equally scattered. No organ showed any marked degree of increase in connective tissue, though a few showed apparently newly formed bile-ducts.

Forty-two organs studied for the presence of the tubercle bacillus gave a positive result in 41 per cent.

Citing a few authorities as regards the relative frequency of tuberculosis of the liver, Barthez and Rillet1 found tubercles in the liver in 71 out of 312 tuberculous children. Willigk² in 476 autopsies of both adults and children affected with tuberculosis found the liver involved in 10. Widl³ found nuclei and the remains of hepatic cells loaded with pigment in softened tubercles, and frequently observed the tubercles colored vellowish in the centre. Frerichs⁴ mentions that tuberculous deposits in the liver at one time assume the form of gray, transparent tubercles, the size of millet seeds, and at another that of yellowish nodules, varying in size from "a lentil to a pea." They may be distributed more or less uniformly throughout the parenchyma, but are most frequently situated beneath the capsule. Softening of these tubercles terminating in the formation of small vomicæ rarely occurs, and is never so extensive as to give rise to local or general derangements, like those which result from pulmonary phthisis. Barthez and Rillet mention a case of cirrhosis associated with tuberculosis reported by Tonnelé, where its existence (tuberculosis) led to the diagnosis of cirrhotic contraction of the liver. Small vomicæ, Frerichs maintains, must not be confounded with cysts which are produced by tuberculous disease of the ducts. condition, recognized by Rokitansky, Barrier, and Barthez and Rillet, is characterized by masses of tubercles deposited around the bile ducts. the channels of which become more and more narrowed.

As regards amyloid changes in the liver, Frerichs⁴ claims that the affection rarely accompanies tubercle of the lungs and intestines.

Ullom,⁵ in a study of the liver in tuberculosis, records results of an examination of 37 cases. Fatty infiltration occurred in 24 per cent., amyloid infiltration in 11 per cent., and only two organs showed an absence of passive congestion. In 30 cases, tubercles were demonstrated

strable in 21 typical and in 9 atypical; 7 showed newly formed bileducts, and in 1 of the 7 there was a coincident increase of connective tissue; tubercle bacilli were observed in only 6 cases.

Pernice⁶ found that the liver in tuberculosis shows certain alterations which resemble those occurring in other infections in man, and consist of a vacuolation of the protoplasm, fragmentation and destruction of chromatin, ending in a complete disintegration of the nucleus and of the cell protoplasm, so that in the place of a cell there remains only a network of residual tissue. The necrotic process is most common about the tubercles and in the neighborhood of arteries and veins, and is more extensive in cases of miliary tuberculosis, which Pernice thinks is due to special bacterial poisons.

No such changes in the liver as those described by Pernice were noticed by the writer. Besides the tuberculous changes described, fatty infiltration was observed in 32.2 per cent., passive congestion was noticed in 52 per cent., and only 12 per cent. showed amyloid infiltration.

The spleen was diagnosticated as tuberculous, macroscopically, in but 2 cases, while amyloid infiltration was recognized 9 times. In 7 instances accessory spleens (spleniculi) were encountered. These varied in size from 5 mm. to 2 cm. in diameter, were mostly globular, usually single, and attached by a short pedicle. Microscopically, 97 per cent. of the spleens showed tubercles, while in 54 per cent. tubercle bacilli were demonstrated. Even the splenculi showed histological tubercles.

In studying tuberculosis of the intestinal tract in this series of cases (62), no lesions of the esophagus, stomach, or duodenum were ever noticed. The ileum, however, was quite frequently involved, as well as the appendix. Three cases of perforation of the small intestine were observed. Thirty-six instances of tuberculosis of the intestines were met with. Of these, there were 16 in which the small and the large intestine were both involved, 9 in which the small intestine and the appendix were diseased, while 11 showed disease of the appendix alone. The lesions in the appendix were either distinct ulcers or miliary tubercles. One appendix presented the appearance of an acute catarrhal process. The remainder (10) were tuberculous, as proved by microscopic findings. In over 25 per cent. tubercle bacilli were found in the appendices examined bacteriologically.

Kelly⁷ considers tuberculosis of the appendix as one of the greatest of rarities, and asserts that it may be doubted whether any well-authenticated case of primary tuberculosis of the appendix has yet been recorded. Fenwick and Dodwell (quoted by Kelly) in 2000 subjects that died of tuberculosis found that the intestine was the seat of ulceration in 500 (56.6 per cent.) out of 883 cases. It was quite exceptional to find the appendix uninvolved when there was disease of the ileocecal region. In 17 of the cases the appendix was the only portion of the intestinal tract that presented ulceration. Whether or not this ulceration was tuberculous was not definitely determined.

Haubold⁸ reports a case of appendicitis in which tubercles were found in the adjacent omentum and the removed organ. No indication of pulmonary involvement was noticed at any time.

A portion of the thoracic aorta and iliac arteries was studied in a number of cases with the idea of ascertaining the effect, if any, of the tuberculous processes upon these structures. In twenty specimens no lesions of any kind were demonstrable, and the study was discontinued.

In making studies of the heart muscle, a portion of the left ventricle was always selected. In not one of the 83 cases was there the least suggestion of tubercle formation. Occasionally a small accumulation of polymorphonuclear leukocytes and lymphoid cells could be seen between the fibers, but at no time were giant cells or caseation encountered. In some sections fatty infiltration was present, and in other sections what appeared to be a fragmenting process of the myocardium was observed, as well as a beginning fibrosis. Organisms that resembled the tubercle bacillus morphologically and tinctorially were demonstrable in 8 specimens. They were quite few in number, and only by examining several sections could two or three bacilli be made out. It seems from the histological study of this organ, in this series, that the only apparent lesion is the seeming fragmentation of some of the fibers and a slight fibrosis.

In a study of the contents of the thoracic duct to determine the presence of tubercle bacilli, 22 cases were studied. The technique was as follows: The duct was dissected from the receptaculum chyli to the point of emptying into the subclavian vein. Both ends were

ligated, the duct thoroughly washed in ordinary tap water, then with sterile water, and securely fastened to a block of cork and mopped thoroughly with a small piece of absorbent cotton. With a sterile hypodermic syringe the vessel was punctured and the fluid contents withdrawn. Usually but one or two drops of fluid was all that could be obtained. Spreads were then made in the ordinary way, and stained by the usual technique for tubercle bacilli. In nearly all the spreads the cellular elements were few. In some, cells resembling lymphocytes were very common, while in a few, cells resembling endothelial cells were present. Only in an occasional preparation was a polynuclear leukocyte observed. All of these 22 cases were of the chronic pulmonary variety, with the exception of 1 case of acute miliary tuberculosis.

In 16 of the cases, or 72.7 per cent., tubercle bacilli were recognized. In most of the positive cases but one or two organisms were found, and these were found only after a most patient and careful search, lasting in most instances considerably over an hour. Only in 1 case was a distinct clump of organisms seen, and this clump consisted of more than twenty bacilli. In not one of these cases was tuberculosis of the duct present, not even the slightest degree of thickening being evidenced anywhere along its entire length. In several, histological examination showed some small accumulations of lymphoid cells, but none resembling in any respect true tubercles.

Longcope, in 25 cases of tuberculosis in which the process was more or less generalized, found 17 typical instances of generalized acute miliary tuberculosis. In 12 of these 17 cases the thoracic duct showed a more or less extensive tuberculous process. In 1, while there was no tuberculosis of the wall of the vessel, many tubercle bacilli were found in smears from the duct lymph. In 1 of the 4 remaining cases in which the duct was normal, there was a primary disease of one epididymis and testicle, with organized thrombi in the vesical veins containing caseous masses and tubercles. Of the 23 cases of generalized tuberculosis, 15, or over 65 per cent., showed disease of the duct, or, as in one instance, tubercle bacilli in the lymph from the duct. Of the acute cases, in over 76 per cent. the duct was affected. In 6 instances the generalized process was subacute or chronic, and while large tubercles or caseous masses were scattered

in small numbers through the various organs, in only 2 was there tuberculosis of the thoracic duct. In both the tubercles were small and occurred sparingly, but in 1 a few tubercle bacilli were found in smears from the duct. In 2 other cases the tuberculous process was of a chronic character, and confined to the lungs and peritoneum. Both of these cases presented normal thoracic ducts.

Longcope's conclusions are, that tuberculosis of the thoracic duct is of great frequency in cases of acute generalized tuberculosis; and in certain cases of the acute generalized disease, tubercle bacilli may be found in the lymph from the duct, though the duct itself is not the seat of tuberculosis. In a small percentage of cases of subacute or generalized tuberculosis the duct may also be affected. Quite a number of observers—Weigert, Koch, Stilling, Benda, Brasch, and others—quoted by Longcope, have found tuberculosis present in the thoracic duct in cases of acute miliary tuberculosis.

Silbergleit, 10 in a number of cases of tuberculosis gone over for four years, claims that in 95 per cent. tubercles of the vessels or of the thoracic duct were found which had permitted the bacilli to gain the blood stream.

In conclusion, I wish to thank Dr. L. J. Spivak and undergraduate W. C. Wood for assistance in sectioning and staining the different organs.

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Lipoma of Uterus.

By A. G. ELLIS, M.D.

(From the Laboratories of the Jefferson Medical College and Philadelphia Hospital.)

The uterus is an organ very frequently invaded by new-growths. Williams, in a study of 13,824 patients of both sexes treated in four large London hospitals because of primary neoplasms, found that 2649, or 19.2 per cent., of the tumors were of uterine origin. Of these patients 9227 were women, and 28.7 per cent. suffered from tumor of the uterus. Of the 13,971 tumors analyzed by Gurlt, at Vienna hospitals, 4115, or 29 per cent., were uterine in location. As to the variety of tumor most frequently found in this organ, Williams' series, which may be taken as fairly representative, was made up as follows: cancer, 1571; myoma, 883; non-myomatous polyp, 191; sarcoma, 2; cystoma, 2. In this series there is no instance of the type of tumor I report, and other similar statistics attest the rarity of this growth.

According to Seydel's² findings, in 1903, only 4 cases of true lipoma of the uterus have been reported, and of these only 1 compares in size and location with the present specimen. Two of the 4 were tiny, cervical polyps and a third was a subserous growth the size of a cherrystone. In addition to these 4 cases of lipoma there are on record 2 of lipomyoma and 4 of lipofibromyoma. Still another, in Lebert's Atlas, is designated simply as adipose tissue. Seydel accepts as authentic cases of lipomatous tumors of the uterus only these 11, rejecting 3 cases cited by Knox because they were not examined microscopically. Although at least 2 of the latter appear, from their gross description, to have been lipomas or lipofibromas, Seydel for the reason given is probably justified in excluding them from his list. The cases collated and reported by him are briefly as follows: I have verified all but 2, which I made no attempt to trace.

Lebert, in his Atlas of Pathologic Anatomy, 1857, pictures a uterus containing "adipose tissue." The illustration shows clearly a lobulated fibro- or musculo-fatty tumor embedded in the uterine wall.

In 1880 Stroinski reported finding a small polyp on the anterior

lip of the cervix of a woman, aged twenty-eight years, who had been sterile, presumably because the growth acted as a valve to the canal. Removal of the polyp showed it to be a lipoma, and pregnancy subsequently occurred.

Orth, in 1893, makes bare mention of a cervical polyp, the size of an almond, which proved to be a lipoma.

Brünnings, in 1899, reported a case of lipomyoma, the size of a child's head, in the anterior wall of the uterus of a woman, aged fifty-five years. The tumor was interstitial in location.

Franque, in 1901, described a lipofibromyoma in a woman, aged fifty-four years. The growth was located as a cervical polyp the size of a pigeon's egg.

Knox,³ in 1901, described in detail a lipomyoma, 10 x 13 x 10 cm. in size, located interstitially in the posterior wall of the uterus of a woman, aged sixty-two years. The symptoms and the findings at operation (hysterectomy) led to the diagnosis of uterine myoma. Further study showed the true nature of the growth.

Merkel, also in 1901, reported two cases. One was an interstitial lipoma, the size of a billiard ball, located near the right tube. The other was a lipofibromyoma the size of an orange occupying the right half of the body of the uterus of a woman, aged sixty-three years.

Jacobson, in 1902, described a lipofibromyoma found in a patient, aged sixty-eight years. The tumor was 8 cm. in diameter and was situated toward the right side in the anterior wall of the body of the uterus.

Seydel, in 1903, found a walnut-sized lipofibromyoma in the uterus of a woman, aged fifty-eight years. The specimen was an interstitial growth in the fundus.

In commenting on Seydel's case, Meyer reports in the same paper a lipoma the size of a cherry-stone found in a patient, aged forty-two years. This nodule was subserous in location, in the fundus.

The 3 cases mentioned by Knox and not accepted by Seydel are the following:

Lobstein (cited by Meckel), in 1803, described a "fatty tumor" of a uterus which enlarged the organ to the size of a seven or eight months' pregnancy.

Seegar, in 1853, reported a case occurring in a widow, aged fifty-

three years. A tumor the size of a child's head protruded externally and was removed by ligation of the pedicle. Macroscopically it was a fatty tumor traversed by firm fibrous bands.

T. Smith, in 1861, showed to the Pathological Society of London a specimen from a woman, aged forty years. A pedunculated mass, the size of two fists, projected from the fundus, and proved to be largely firm fibrous tissue. Embedded in it and easily shelled out was a fatty tumor the size of a pigeon's egg.

I have found no cases in addition to those mentioned, hence the number of undoubted cases of fatty tumors of the uterus appears to be but 11, to which the 3 just mentioned should possibly be added. Several cases reported by the older writers were clearly instances of fatty degeneration of preëxisting tumors, usually myomas. Even this change is rare, McDonald⁴ stating that of 530 cases of uterine fibromyoma reported by Cullingworth, Scharlieb, and Frederick only 7 showed fatty degeneration. McDonald found no instance in 280 specimens nor did Noble⁵ in 278.

The specimen forming the basis of this paper was an autopsy finding in a woman, aged sixty years, who died in the Philadelphia Hospital July 19, 1906. Her family and previous medical history are recorded as negative; the ailment for which she was admitted, and which finally caused her death, was cerebral apoplexy. I am indebted to Dr. William Pickett for permission to publish the case. The pathological diagnosis included fatty heart, arteriosclerosis, chronic endocarditis, and contracted kidneys.

The uterus exhibited a globular swelling occupying the fundus and anterior portion of the body of the organ. This was much softer than is usual with uterine tumors, but was regarded as a soft myoma with possibly degenerative changes in the interior. A median incision through the organ revealed the growth to be a mass of yellow fat showing well the lamellar arrangement common to many lipomas. Spaces produced by partial separation of some of these may be seen in the gross specimen. The only suggestion of anything other than adipose tissue in the growth was at one point near the periphery where an irregular area slightly more than 1 cm. in extent appeared calcareous. Preservation of the specimen by the Kaiserling method has caused this area to become essentially as soft as the surrounding

fat. The tumor measured 8 cm. in vertical and 6.5 cm. in transverse diameter. It was interstitial in location, the muscle between it and the endometrium averaging 0.5 cm. and that external 1 cm. in thickness. The growth was easily separated from the enclosing muscle and possessed what appeared to be a thin grayish capsule, though this structure could readily be detached from both the tumor and the uterine wall.

Microscopically the tumor shows the structure of a typical lipoma. The fat cells are round or polygonal in outline and for the most part much larger than those in normal adipose tissue. The nuclei are crowded to one side and flattened. The bloodvessels are not numerous and are well developed. The tumor possesses but little fibrous tissue except at points beneath what corresponds to the capsule where there are irregular bands passing for a little distance into the adipose tissue; none of them are broad and all are quite cellular. Bordering the tumor is what may be regarded as a capsule of fibrous or fibro-This is more dense in the shape of a narrow band immediately surrounding the tumor proper, though even this is quite cellular and contains numerous small vessels. At points this band forms the entire boundary, but for the most part there is external to and continuous with this a network of fibrous and adipose tissues from two to three or four times the width of the described band. As a rule the adipose tissue predominates in this outer zone of the capsule, in some areas being conspicuously in excess. Over part of the extent a very narrow band of fibrous tissue forms the outer limit of this zone, but at many points almost pure adipose tissue constitutes the margin of the sections. In the tumor itself no muscle is present. At points in the capsule there is a faint resemblance to greatly attenuated muscle fibers, but, if they are such, which I doubt, they are so altered as to render identification impossible. The tumor, therefore, is a pure lipoma.

Lipomas of the uterus are of pathological and scientific rather than of clinical interest, being found accidentally at operation or at autopsy. As with most tumors, the exciting cause is unknown. The ages of the patients in the series varied from twenty-eight to sixty-eight years. That activity of the uterus has no bearing on the question may also be inferred from the fact that several of the women were sterile while one had a record of thirteen normal labors and three miscarriages.

As the normal uterus contains no adipose tissue, the histogenesis of these tumors is their most interesting feature. Their rarity is an evidence that unusual conditions obtain when they do occur. Knox considers them as supporting, in a limited way, the theory of Cohnheim regarding the histogenesis of tumors. Brünnings believes that muscle cells change into fat cells but Merkel failed to demonstrate that these cells change by substituting fat droplets for their protoplasm and believes that such metaplasia is not possible. He prefers to regard the tumors as due to hyperplasia of embryonal fat in the uterus. Franque ascribes the origin of the fat to the connective-tissue elements of the uterus, and Jacobson favors the view that similar elements in myomas become fat cells. According to his view, lipofibromyomas arise from fibromyomas by fatty infiltration of the latter.

Sevdel describes degenerative changes in the cells of the stroma. but could not trace the formation of fat cells from either muscle or connective-tissue elements. He regards, as the easiest explanation, the view that these tumors spring from lipoblasts dislocated during an early period of development. Lipoblasts are not found normally around the small branches of vessels, but they may be carried there from the larger trunks, as Mever states that he has seen fat from the parametrium extend into the substance of the uterus along with bloodvessels, but never to any great depth. Seydel is not an extreme partisan of this view. Under such circumstances both elements of a lipomyoma might develop from the walls of vessels if we accept the view that myomas arise from the muscle coat of these structures. Sevdel, however, found no relation between the muscle of the tumor and the walls of the bloodvessels. As none of these or other advanced theories have been proven, it can only be said that the histogenesis of uterine lipomas is at present undetermined.

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September 27, 1906.

Method for the Determination of B. Typhoid and B. Coli Communis in Water and Milk

By H. J. SOMMERS, JR., M.D.

THE problem of isolating the bacillus of typhoid fever from water, milk, etc., has occupied the attention of many bacteriologists, and a number of methods have been presented. All, so far as I am aware, have followed the same general rule of using some acid to retard the growth of or kill all other organisms than B. typhosus or allied germs, and recommend the use of from 1 to 10 drops of the suspected liquid in about 10 c.c. of culture medium. Thus, Ufflemann, in 1891, recommended that 8 drops of a 5 per cent. solution of citric acid be added to 10 c.c. of gelatin tinged with methyl violet. He claimed that typhoid colonies present a deep-blue color and can easily be recognized. Dunbar, however, proved that B. typhosus cannot live in a medium containing citric acid in the strength recommended by Ufflemann, but that B. coli communis readily develops. Holtz, in 1800, recommended potato gelatin, acidified with 0.05 per cent. carbolic acid. Parietti in the same year recommended the use of a fluid as follows: Carbolic acid, 5 grams; hydrochloric acid, 4 grams; distilled water, 100 grams.

He recommended that three tubes, each containing 10 c.c. bouillon, be inoculated with from 1 to 10 drops of suspected water, and 3, 6, and 9 drops of the above fluid added to each of the tubes; at the end of twenty-four, forty-eight, and seventy-two hours plating is done.

Vincent, in 1890, proposed that water be introduced into a test tube containing sterile peptone bouillon, and 5 drops of a 5 per cent. solution of phenol added and the tube incubated; these methods, together with a number of others, are similar, and all result more or less frequently in failure.

In my opinion the quantity of suspected material used in such tests is often inadequate. The proof of this may be obtained from almost any bacteriologic laboratory where such examinations are made. In the laboratory of the Bureau of Health of Philadelphia I understand that upward of 2000 samples of milk were examined for B.

typhosus, all proving negative; but B. coli communis was found in many.

Being called upon to determine the source of typhoid infection in the State Hospital for the Insane at Norristown, during August, September, and October of 1905, I resorted to all of the usual methods, making several hundred inoculations, and failed to find B. typhosus, but in some instances found B. coli communis. After repeated failures, and not being able to learn of any methods other than those already used, and after giving much thought to the subject, it occurred to me that, in the first place, milk is an ideal culture medium for B. typhosus, and that Parietti's fluid in definite quantities destroys most bacteria, but leaves B. typhosus and allied organisms alive; and that, therefore, it might be advantageous to take suspected milk itself as the culture medium and to destroy most of the ordinary germ life with Parietti's fluid.

Sterile nutrient bouillon to the amount of 300 c.c. was placed in sterile flasks, infected with typhoid cultures, and various amounts of Parietti's fluid added to each flask, 2.5 c.c. up to 4 c.c. Pure cultures of B. typhosus were obtained from each. Four flasks were infected. and 4.2, 4.6, 4.6, and 5 c.c. of Parietti's fluid added, respectively; a culture was obtained from the flask containing 4.2 c.c. Parietti's fluid, the remainder being sterile. Three additional flasks were prepared and received 4.3, 4.4, and 4.5 c.c. of Parietti's fluid, respectively, and cultures were obtained from each at the end of forty-eight hours. This showed that the limit of use of Parietti's fluid was 4.5 c.c. to each 300 of liquid used. Experiments were then made with sterilized milk infected with a known B. typhosus, and after repeated trials the following method was adopted: 300 c.c. of suspected milk was introduced into each of three sterilized flasks, to which were added 3.4, 4, and 4.4 c.c. of Parietti's fluid, respectively; they were well shaken and the flasks placed in the incubator at 40° C. At the end of twenty-four, forty-eight, and seventy-two hours, culture plates were made from each, and typical colonies of B. typhosus were found in one sample of suspected milk. Several other trials confirmed this, and pure cultures were carried through the various media, tested for indol and phenol, and Widal reactions were made with all suspected cultures. After demonstrating to my own satisfaction that I had

found B. typhosus in the milk, water from the farm from which the infected milk was brought was obtained, and the same method was used, except that a concentrated bouillon was made and added to the water to give proper nutrient material for the growth of germ life; here also B. typhosus was found.

Subsequently it was found that the dilution of the suspected milk with about an equal quantity of boiled distilled water gave good results, and, further, that if an Erlenmeyer flask is used for this culture it should not be over one-half full, as the cream rising to the top thickens and is not readily broken when the culture is shaken to obtain material for plating unless ample room is left in the flask.

Failures in a number of cases during practical examinations where I had every reason to believe B. typhosus should be found caused me to make all first cultures anaërobic, which, of course, eliminated all aërobes with so much more chance of success; and anaërobic culture as a routine in this work is recommended.

Since the discovery of the above method, Starkey published an article in which he described an original method for the isolation of B. typhosus and B. coli communis, this method depending on the motility of the germ. He used a labvrinth of glass tubing with a bulb at one end, with tubulares at the upper curvature of each limb of the labyrinth. He fills the apparatus with bouillon, acidulates with 0.05 per cent. pure carbolic acid, the openings are plugged with cotton-wool, and the whole sterilized. Inoculations are made at the bulb end, and the apparatus is then subjected to perfect anaërobic conditions and placed in the incubator. At the end of twentyfour to forty-eight hours, I c.c. of the fluid is removed from the individual tubulares, beginning with the one nearest the bulb, and a plate made of each. He found that the typhoid bacillus travels faster and farther than the colon bacillus, and is usually found one or two tubes ahead of the colon bacillus. In this way he was always able to obtain pure cultures of typhoid bacilli at the end of fortyeight hours.

Desiring to simplify my method and to do away with the large amounts of plating necessary, I determined to combine the two

¹ American Journal of the Medical Sciences, July, 1906, vol. cxxxii, p. 109.

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methods, and to that end had a labyrinth constructed following Starkey's plan, but in more compact form, which allows the use of a Novy anaërobic jar. The labyrinth was filled with bouillon acidulated with Parietti's fluid in the proportion of 4 c.c. to each 300 c.c. of fluid, and the whole sterilized. After cultivation for twenty-four to thirty-six hours of the suspected milk or water by the method that I have described, about 25 c.c. of the culture was added to the almost completely filled Starkey labyrinth, and as a result pure cultures of B. typhosus were readily obtained from the third or fourth tubulare at the end of twenty-four or thirty-six hours.

Note.—The Proceedings of the Society for 1907 will be omitted and will appear in the special volume to be published in commemoration of the Fiftieth Anniversary of the founding of the Society.

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Surgeon-General's Catalogue.

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